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人工种植天麻的影响因子优化研究*

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摘要：天麻是一种名贵的中草药，可用于治疗多种疾病。自然条件下，天麻通过与真菌蜜环菌的共生从腐木获取营养进行生长。为了对人工大规模种植天麻的影响因子进行优化研究，利用正交设计方法，在昭通小草坝地区开展了一个样本量较大的种植实验。在测试的 4 种因子中，接种蜜环菌对种植天麻影响最大；其次，在露天灌木丛地种植的天麻产量明显高于在林地下的种植。此外，开展了 12 个树种对蜜环菌生长以及天麻种植的影响研究。结果发现十齿花（*Dipentodon sinicus*）对蜜环菌的生长最好，同时，也在天麻种植实验中有最高的产量；然而，添加磷、钾元素并不提高天麻的产量。

关键词：天麻；蜜环菌；人工种植；生态因子；菌材
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Study of Factors for Cultivating the Orchid Species *Gastrodia elata*, a Traditional Chinese Medicine

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Abstract: The orchid speices *Gastrodia elata* is a valuable traditional Chinese medicine and has been widely applied for treating a variety of diseases. The yield of wild *G. elata* is very limited since its vegetative growth is exclusively dependent on its symbiosis with the fungus *Armillaria mellea*, from which *G. elata* is able to obtain nutrients from rotten wood in the forest. To develop a standard for cultivating *G. elata* in large quantities, four factors that may influence the yield of *G. elata* need to be further investigated, including cultivation environment, inoculation volume of *Armillaria*, wood from different tree species, and the effect of fertilizer on the maturation of *G. elata*. To optimize these factors, a large-scale orthogonal experiment was performed in the farmland of Xiaocaoba, Zhaotong, Yunnan Province. Among the four factors tested, inoculation of *Armillaria* played the most important role in the maturation of *G. elata*. The yield of mature tubers, in terms of both tuber weight and quantity, on open ground is greater than that in forested areas. Of the 12 tree species tested, *Dipentodon sinicus* stimulated the largest amount of growth of *Armillaria* and produced the greatest yield of *G. elata* in the farmland. In comparison to the other factors tested, fertilizer showed no effect on the yield of *G. elata*.

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Key words: *Gastrodia elata*; *Armillaria*; Cultivation; Ecological factor; Wood

Gastrodia, the tuber of the orchid species *Gastrodia elata* Blume, is a valuable traditional Chinese medicine and has been widely applied for treating a variety of diseases, including headaches, dizziness, hemiplegia, rheumatism, and epilepsy (Tang and Eisenbrand, 1992). Recently, the active compounds from this tuber have been found to act as anticonvulsive agents, antioxidants, and antidepressants and may also be potential treatments for Alzheimer's disease (Zhou *et al.*, 2006; Chen *et al.*, 2008, 2009; Chen and Sheen, 2011). Several important, medically-relevant biological activities of Gastrodia have been demonstrated, such as anti-cancer (Heo *et al.*, 2007) and anti-asthma properties (Jang *et al.*, 2010). In addition, Gastrodia has also been considered safe for long-term administration, and therefore it has previously been used to improve the health and prolong the life of humans (Chen and Sheen, 2011).

In nature, *G. elata* is a perennial herb and usually grows on the edge of or within the forest (Zhou, 1973). Because *G. elata* is achlorophyllous and aphyllous, it has to obtain nutrients by forming symbiotic relationships with two types of fungi: *Mycena osmundicola* and several species from *Armillaria*. While the former fungus stimulates the germination of *G. elata* seed, the latter is thought to decompose the wood from dead trees and thereby release into the soil the nutrients necessary for the vegetative growth of *G. elata*. Only in the presence of these two fungi can *G. elata* complete its life cycle from seed to protocorm to immature tuber to mature tuber (Xu *et al.*, 1989). Of the *Armillaria* species, *A. mellea* is the first reported fungus to form a symbiotic relationship with *G. elata* (Kusano, 1911) and has been considered to play the most important role in the maturation of *G. elata* (Anderson and Ullrich, 1979; Xu and Guo, 2000; Wang *et al.*, 2007).

The yield of wild *G. elata* has traditionally been very low due to the lack of appropriate methods for improving its cultivation. In 1968, a methodology for cultivating *G. elata* was invented and applied, and since then its yield has been greatly improved. The current cultivation process begins by first placing several layers of fresh wood within a hole in the ground. Naturally-collected tree bark and branches, which are infected with *Armillaria*, are then inoculated and cultivated for nearly two months. Subsequently, immature tubers of *G. elata* are planted and then cultivated after an additional year to obtain the mature tubers (Zhang and Yang, 2007).

While this cultivation process has been used for many years, several problems still need to be solved in order to develop a reliable standard for cultivating *G. elata* in large quantities. First, although *G. elata* has been commonly cultivated both in the forest and on the open ground, it is unclear if the yield of *G. elata* differs among these two types of environments, which can greatly vary in temperature, humidity, and amount of sunlight. Second, inoculating *G. elata* with commercial *Armillaria* has been widely used to increase the yield and shorten the production cycle of *G. elata* (Hua, 2004); however, the optimal quantity of *Armillaria* to use for inoculations has not been determined. Third, *Armillaria* species are able to grow on over 600 plant species, including trees, shrubs, and herbs. In practice, while farmers usually use a mixture of wood from different tree species for cultivating *G. elata* (Zhang and Yang, 2007), it remains to be determined how different types of wood affects the growth of *G. elata*. Finally, it is unclear if there are any positive effects of fertilizer on the yield of *G. elata*. In this study, we use an orthogonal design to perform the first large-scale *G. elata* cultivation experiment to identify the most important factors for improving the yield, i. e. the growth and weight of *G. elata*.

1 Materials and methods

1.1 Description of experimental site

Cultivation of *G. elata* was performed in Xiaocaoba, Zhaotong, Yunnan Province of China (at approximately 27°N and 104°E, with an average altitude of 1 700 m). Xiaocaoba is famous for the production of the highest quality of *G. elata* specimens in all of China, which is due, in part, to the climatic suitability of this area. Xiaocaoba receives an annual rainfall of 960-1 300 mm and is characterized by relatively high humidity (ranging from 76 to 85% each year) (Zhang and Yang, 2007). The average temperature is above −1 ℃ in the coldest month and is below 25 ℃ in the warmest month (Yuan *et al.*, 2002).

1.2 Effects of tree species on the growth of *Armillaria*

In total, twelve tree species, which are commonly used for the cultivation of *G. elata* in Xiaocaoba, were selected to determine how the selection of tree species influences the growth of *Armillaria*. These tree species we chose included: *Malus domestica*, *Sorbus pohuashanensis*, *Glochidion puberum* (Linn.) Hutch, *Dipentodon sinicus* Dunn, *Padus brachypoda* (Batal.) Schneid, *Illicium verum*, *Phoebe zhennan* S. Lee et F. N. Wei, *Betula* sp, *Schima superba* Gardn et Champ, *Cerasus serrula* (Franch.) Yüet Li, *Acer oliverianum*, and *Lithocarpus balansae* Bl.

Tree branches were freshly collected from the local forest, brought back to the laboratory, and cut into fragments of 0.5 cm (long) ×0.5 cm (wide) × 0.1 cm (high). About ten grams of each wood fragment was boiled in water for 30 minutes, producing 100 mL of leaching solution. Glucose (2%) and agar (1.8%) were then added to the leaching solution to make the medium for culturing *Armillaria*. For each tree species, we created 5 replications of this culturing medium. After 10 days of culturing at 25 ℃, the length of the hyphae of *Armillaira* was measured and used as an index of growth of the *Armillaria*.

1.3 The cultivation of *G. elata*

Typically, it takes three years to obtain mature tubers from the seeds of *G. elata*. In the first year, seeds germinate with the help of the symbiotic fungus *M. osmundicola* and grow into protocorms. The protocorms are then used to obtain immature tubers after one year of cultivation with *Armillaria*. Finally, the immature tubers develop into mature tubes after cultivation with *Armillaria* for an additional year. Rather than waiting for tubers to grow and mature from seed, we acquired commercially-developed immature tubers that we then propagated and used to cultivate mature tubers.

An orthogonal experiment was performed to evaluate the effects of wood type, inoculation volume of *Armillaria*, and fertilizers on the production of *G. elata* (Table 1). Each of these factors had three levels, resulting in 9 unique combinations of factors. Based on the results from the *Armillaria* growth experiments, only the best tree for the growth of *Armillaria*, *Dipentodon sinicus*, and of the seven worst trees for *Armillaria* growth, only *Sorbus pohuashanensis* and *Betula* sp., were chosen and collected from the local forest for the *G. elata* cultivation experiments. Commercially-produced *Armillaria* M1 was purchased from Zhaotong Shengnong Limited, and we used three inoculation volumes (0, 250 and 500 g per hole) for each type of wood. In some cases, fertilizer (10 g/hole) were added and completely mixed with soil when planting *G. elata*.

Table 1 L_9 orthogonal array applied in the cultivation of the *G. elata*

Factor	1	2	3	4	5	6	7	8	9
Type of wood [a]	D	D	D	S	S	S	B	B	B
Inoculation volume of *Armillaria* /g·hole	0	500	250	250	0	500	500	250	0
Fertilizer	K_2SO_4	KH_2PO_4	—	KH_2PO_4	—	K_2SO_4	—	K_2SO_4	KH_2PO_4

[a] The type of wood. D, *Dipentodon sinicus*; S, *Sorbus pohuashanensis* and B, *Betula* sp

In September 2010, a total of 540 holes, each 0.5 m (wide) × 1 m (long) × 0.5 m (high), were dug, half on the open ground and half in the forest. At both of these environments (i.e. ground and forest), each orthogonal test was arranged with 9 treatments (see above) of 30 replicates per treatment. In each hole, 5−10 freshly-cut wood fragments (about 10 kg in total) were laid in two layers, with *Armillaria* M1 as needed. The holes were then filled with soil and *Armillaria* was cultivated for two months. In November 2010, the top soil of each hole was removed, and the immature tubers of *G. elata* were planted on top of the layers of wood. Fertilizers were applied as needed, and then we refilled the holes with the soil. About one year later, in October 2011, all of the mature tubers were collected, counted, and weighed.

1.4 Data analysis

All data were analyzed using the software package SPSS13.0 (Spss, Inc., 2004). One-way analysis of variance (ANOVA) was performed to determine the effect of tree type on the growth of *Armillaria*, and compare the effects of each factor on tuber weight in the orthogonal test previously described (Deng *et al*., 2009). The weight and number of tubers obtained from the forest and from the open ground was compared using a nonparametric independent-sample test.

2 Results

2.1 Effect of wood type on the growth of *Armillaria hyphae*

The tree species *D. sinicus* stimulated the greatest amount of *Armillaria* growth (mean length of 3.34 cm for the rhizomorph), which was significantly higher than for almost all of the other tested wood types ($P < 0.05$). On the other hand, the tree species *S. pohuashanensis* and *Betula* sp displayed the weakest effects on the growth of *Armillaria*, with the length of the rhizomorphs growing less than 2 cm (Fig. 1).

2.2 Cultivation of *G. elata*

Among the four tested factors, inoculation of *Armillaria* plays the most important role in determining the weight of the tubers ($F = 25.706$, $P < 0.001$). The type of field environment (i.e. open ground vs within the forest) was the second most important factor influencing the yield (in terms of tuber weight) of *G. elata* ($F = 19.141$, $P < 0.001$). In addition, the type of wood also significantly affected the weight of tubers ($F = 6.058$, $P = 0.003$). In contrast, the addition of fertilizers had no effect on the yield of *G. elata* (Table 2).

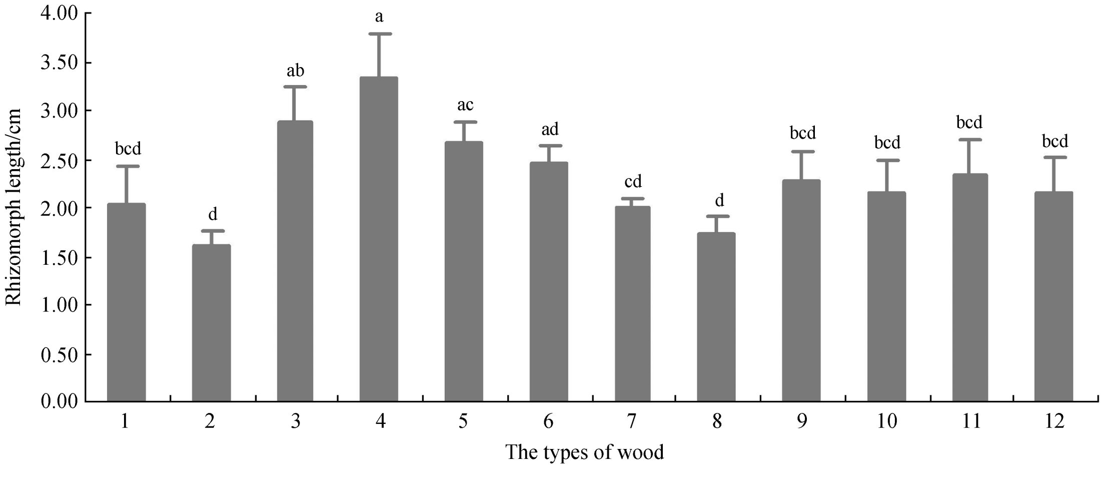

Fig. 1 Effects of the leaching solution of different tree woods on the growth of *Armillaria*.
The bars labeled with different letters are statistically significant ($P < 0.05$)
1. *Malus domestica*; 2. *Sorbus pohuashanensis*; 3. *Glochidion puberum*; 4. *Dipentodon sinicus*; 5. *Padus brachypoda*; 6. *Illicium verum*; 7. *Phoebe zhennan*; 8. *Betula*. sp; 9. *Schima superba*; 10. *Cerasus serrula*; 11. *Acer oliverianum*; 12. *Lithocarpus balansae*

Table 2 Statistics of the effects of four factors on the weight of mature tubers of *G. elata*

Source	Type III Sum of Squares	*df*	Mean Square	*F*	*Sig.*
Inoculation volume of *Armillaria*	2.565	2	1.282	25.706	0.000
Field environments	0.955	1	0.955	19.141	0.000
Type of tree woods	0.604	2	0.302	6.058	0.003
Inorganic elements	0.153	2	0.077	1.534	0.217
Error	23.696	475	0.065		
Total	40.398	483			
Corrected Total	27.976	482			

The performance of individual factors on influencing the weight of tubers is showed in Fig. 2. Inoculation of *Armillaria* significantly affected the weight of the tubers, in an inoculation volume-dependent manner, i. e. greater inoculation volumes resulted in greater tuber weights. The use of wood from the tree species *D. sinicus* significantly increased the weight of the tubers, nearly doubling the weight of the tubers when compared to using *Betula* sp. ($P < 0.05$).

The yield of mature tubers, in terms of both the weight and the quantity produced, on the open ground is significantly greater than that from within the forest ($P < 0.05$). On average, each hole from the open ground environment yielded 3.2 tubers of *G. elata*, weighing an average of 0.209 kg, which was nearly two times the tuber yield produced from within the forested environment (1.8 and 0.114 kg, respectively; Fig. 3).

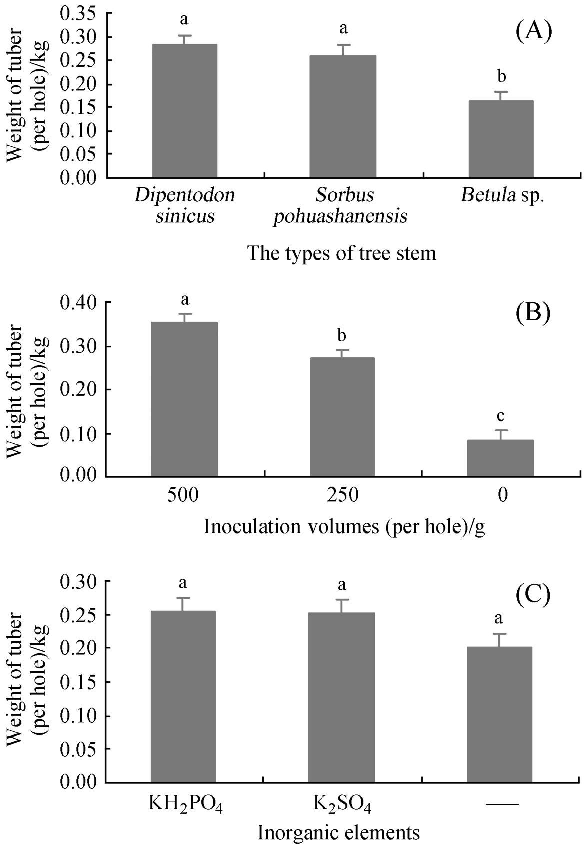

Fig. 2 Effect of woods from different tree species (A), inoculation volume of *Armillaria* M1 (B) and inorganic elements (C) on the tuber weight of *G. elata*. The bars labeled with different letters are statistically significant ($P < 0.05$)

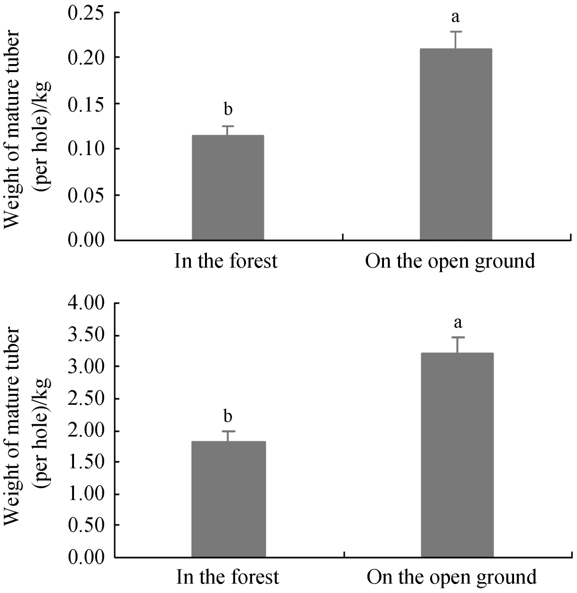

Fig. 3 Comparison of the yield of the *G. elata* cultivated in the forest and on the open ground. The bars labeled with different letters are statistically significant ($P < 0.05$)

3 Discussion

The vegetative growth of *G. elata* primarily depends upon the nutrients supplied by the decomposition of wood by the fungus *Armillaria*, which in turn parasitizes the plants. Therefore, the wood is a primary nutrient source for both *G. elata* and *Armilllaria* (Zhang and Yang, 2007). Although *Armillaria* is able to infect a variety of tree species (Sun *et al.*, 2007), different trees are chemically distinctive and could therefore differentially affect the growth of *Armillaria* and consequently the production of *G. elata* (Rong and Cai, 2010). In general, the faster and more branched the *Armillaria* hyphae, the higher the yield of *G. elata* (Wang *et al.*, 2001). This study confirmed that the type of wood does impact the growth of *Armillaria* and suggested that, of the 12 tree species studied, *D. sinicus* has the greatest effect on increasing the hyphae growth of *Armillaria* (Fig. 1). Similarly, *D. sinicus* also demonstrated a greater ability to increase the weight of tubers in the cultivation of *G. elata* than the two other tree species: *S. pohuashanensis* and *Betula* sp. (Fig. 2). Currently, local farmers cultivate *G. elata* using a mixture of wood from different tree species, primarily selecting from among the 12 wood types used in this study (Fig. 1). This method not only causes the low and unstable production of *G. elata*, but also results in the unnecessary damage of local forests resulting from the poor utilization of appropriate wood types. Based on our results, we recommend that people involved in the cultivation of *G. elata* plant the tree species *D. sinicus*, which will not only increase the yield of *G. elata* for the farmers, but also slow down the destruction of local forests.

The yield of tubers inoculated with the *Armillaria* fungus is significantly greater than the yield without any inoculation (Table 2). Moreover, our results indicate that larger inoculation volumes of *Armillaria* produce higher yields of *G. elata* (Fig. 2). However, caution should be taken when using a large inoculation volume of *Armillaria* since too much *Armillaria* might negatively impact the growth of *G. elata* (Zhang and Yang, 2007). Aside from the primary nutrition provided by *Armillaria*, *G. elata* has also been thought to be able to obtain partial secondary nutrition, e. g. phosphorous uptake, from the soil (Zhuang *et al.*, 1983). However, our results demonstrated that inorganic elements have no impact on the yield of tubers (Table 2, Fig. 2). This could be because inorganic elements in the local soils are sufficient for the growth requirement of *G. elata*. Therefore, the addition of chemical nutrients to the soils for improving the cultivation of *G. elata* is not recommended.

The growth of *G. elata* depends upon optimal light, temperature, and humidity conditions (Zhang and Yang, 2007), which vary among forested areas and open ground. In this experiment, the total weight of mature tubers cultivated on the open ground was nearly two times of that produced from the forested environment, where the temperatures were much cooler (Fig. 3), thus supporting previous reports that the growth of *G. elata* benefits from high temperatures (Liu and Wu, 1998). However, more immature tubers were obtained from the forest than from the open ground (data not shown). Since these immature tubers from the forest will take an extra year to grow into mature tubers than those from the open ground, this implies a higher cost of time and land-use for farmers if cultivating *G. elata* from inside the forest.

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